50. (Amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element;

said method comprising providing to said cell an effective amount of a ligand for said modified ecdysone receptor, wherein said ligand is not normally present in said cell; and wherein said ligand is not toxic to said cell.

53. (Amended) A method for modulating the expression of an exogenous gene in an isolated mammalian cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element;

said method comprising providing to said mammalian cell an effective amount of a ligand for said modified ecdysone receptor; wherein said ligand is not normally present in said mammalian cell; and wherein said ligand is not toxic to said mammalian cell.

REMARKS

Courtesies extended to Applicants' representative in the telephonic interview held on February 22, 2001, are acknowledged with appreciation.

The present invention provides methods for modulating expression of exogenous genes in cells containing a defined DNA construct. DNA constructs contemplated herein comprise an

exogenous gene under the control of a (modified or unmodified) ecdysone response element plus a modified ecdysone receptor which, in the presence of an appropriate ligand, binds to the ecdysone response element, and optionally a further receptor which, in the presence of the modified ecdysone receptor, can act as a silent partner. The invention method comprises providing to a cell containing the construct an effective amount of a ligand for the modified ecdysone receptor that is not normally present in the cell. The presence of ligand for the modified ecdysone receptor (and optionally, the presence of a receptor that can act as a silent partner) promotes the formation of ligand-receptor complexes which can interact with invention modified ecdysone response element, thereby modulating expression of the exogenous gene.

Invention methods for modulating exogenous gene expression are useful in a wide variety of applications. Modulation of exogenous gene expression is desirable in numerous cell populations ranging from transiently modified cells to stably transformed cell lines. For example, invention methods can advantageously be employed in *in vitro* cellular expression systems to regulate expression of a recombinant expression product. Similarly, host cells and other recombinant cell types can benefit from invention methods for modulating the expression of an exogenous gene.

Claims 1 to 24, 35 to 42 and 47 to 56 were pending before this response. By the present communication, claims 1, 22 to 24, 50 and 53 have been amended to define Applicants' invention with greater particularity and not in response to any properly citable reference. No new matter is presented by the proposed amendments submitted herewith as all amended claim language is fully supported by Applicants' specification and original claims. The amendments submitted herewith do not require a new search or raise new issues for consideration because they address matters previously at issue during prosecution of the subject application. The proposed amendments are submitted pursuant to the discussion at the personal interview on November 29, 2000 and the follow up telephone discussion held on February 22, 2001. These amendments are respectfully submitted to place the application in condition for allowance. Accordingly, entry of the amendments submitted herewith is respectfully requested.

Upon entry of the proposed amendments submitted herewith, claims 1 to 24, 35 to 42 and 47 to 56 will be pending; the text of these claims as they will read upon entry of the proposed amendments is presented for the Examiner's convenience as Exhibit A.

Rejections Under 35 U.S.C. § 112

Applicants respectfully traverse the rejection of claims 1 to 46 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. As discussed in the personal interview held on November 29, 2000, and further discussed during the telephone conference held on February 22, 2001, all claims are fully enabled (see, for example, Hoppe *et al.*, (2000) *Mol. Therapy* 1:159-164, previously provided for the convenience of the Examiner). However, in the interest of expediting prosecution of the present application and obviating the need to take this application up on appeal, the claims have been amended to direct Applicants' invention to modulation of gene expression in isolated cells (e.g., *in vitro* methods). The amendments to the claims submitted herewith in no way constitute a disclaimer of methods conducted with other than isolated cells. The proposed amendments are submitted herewith in an effort to expedite prosecution of the present application, without prejudice to Applicant's right to pursue claims directed to other applications of invention methods, e.g., gene therapy.

Applicants' invention, as defined by amended claims 1, 22 to 24, 50 and 53, as well as claims dependent therefrom, requires a method for modulating the expression of an exogenous gene in an isolated cell by providing to the cell an effective amount of a ligand (not normally present in the cell) for a modified ecdysone receptor. The cell contains a DNA construct comprising the exogenous gene under the control of an ecdysone response element and a modified ecdysone receptor which, in the presence of a ligand (and optionally in the further presence of a receptor that can act as a silent partner of the ecdysone receptor) binds to the ecdysone response element.

Invention methods to modulate the expression of an exogenous gene in an isolated cell are useful in a variety of systems including *in vitro* expression systems (See, for example, page

8, lines 29-32 of Application specification), cellular expression systems (*e.g.*, page 9, lines 20-21), host cells (*e.g.*, page 10, lines 12-14; and page 39, lines 13-16), mammalian expression systems (*e.g.*, page 35, line 33 to page 36, line 1), recombinant cells (*e.g.*, page 42, lines 1-4 and page 43, lines 32-35), and the like. The specification further provides working examples of invention methods in stable recombinant cell lines (see Examples 3 and 6). Expression of an exogenous gene is modulated *in vitro* in stable isolated recombinant cells containing a modified ecdysone receptor, a heterodimeric partner, and an ecdysone inducible reporter by providing to the cells a suitable ligand such as muristerone or ponasterone.

It is respectfully submitted that the present invention relates, *inter alia,* to methods for *in vitro* modulation of expression of exogenous genes in cells. In view of the ample support provided in the specification for the *in vitro* embodiments of the present invention, and the amendments to claims 1, 22 to 24, 50 and 53 submitted herewith, Applicants respectfully submit that the claims are fully enabled. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph are respectfully requested.

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any issues remain, the Examiner is invited to contact the undersigned at the telephone number set forth below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: 2-28-01

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Enclosure

In Re Application of:
Evans et al.
Application No.: 09/042,488
Filed: March 16, 1998
Exhibit A: Page 1

PATENT
Attorney Docket No. SALK1520-2

EXHIBIT A: CLAIMS AS THEY WILL STAND UPON ENTRY OF THE AMENDMENT

1. (Twice Amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element;

said method comprising providing to the cell an effective amount of a ligand for said modified ecdysone receptor; wherein said ligand is not normally present in the cell; and wherein said ligand is not toxic to said cell.

2. (Reiterated) A method according to claim 1 wherein said modified ecdysone receptor comprises:

a ligand binding domain capable of binding an ecdysteroid;

a DNA-binding domain obtained from a DNA-binding protein; and

an activation domain of a transcription factor,

wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor,

with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an *E. coli* LexA protein.

3. (Reiterated) A method according to claim 2 wherein said modified ecdysone receptor is further characterized as having substantially no constitutive activity in mammalian cells.

4. (Reiterated) A method according to claim 2 wherein the DNA-binding domain of said modified ecdysone receptor is derived from a member of the steroid/thyroid hormone superfamily of receptors.

5. (Reiterated) A method according to claim 2 wherein said activation domain is obtained from a member of the steroid/thyroid hormone superfamily of receptors.

6. (Reiterated) A method according to claim 2 wherein said activation domain is selected from a glucocorticoid receptor activation domain, a VP16 activation domain or a GAL4 activation domain.

7. (Reiterated) A method according to claim 6 wherein said modified ecdysone receptor is selected from VpEcR, VgEcR or GEcR.

8. (Reiterated) A method according to claim 7 wherein said modified ecdysone receptor is VgEcR having the amino acid sequence set forth in SEQ ID NO:5.

9. (Reiterated) A method according to claim 1 wherein said modified ecdysone receptor is present primarily in the form of a homodimer.

10. (Reiterated) A method according to claim 9 wherein said ecdysone response element is the native ecdysone response element.

11. (Amended) A method according to claim 47 wherein said receptor capable of acting as a silent partner is RXR.

12. (Reiterated) A method according to claim 11 wherein said RXR is exogenous to said mammalian cell.

13. (Reiterated) A method according to claim 1 wherein said ecdysone response element is a modified response element which comprises, in any order, a first half-site and a second half-site separated by a spacer of 0-5 nucleotides;

wherein said first half-site has the sequence:

-RGBNNM-,

wherein

each R is independently selected from A or G;

each B is independently selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

each M is independently selected from A or C;

with the proviso that

at least 4 nucleotides of each -RGBNNM- group of nucleotides are identical with the nucleotides at comparable positions of the sequence -AGGTCA-; and

said second half-site is obtained from a glucocorticoid receptor subfamily response element.

14. (Reiterated) A method according to claim 13 wherein said response element has substantially no binding affinity for farnesoid X receptor (FXR).

15. (Reiterated) A method according to claim 1 wherein said ligand is a naturally occurring ecdysone, an ecdysone-analog or an ecdysone mimic.

16. (Reiterated) A method according to claim 15 wherein said naturally occurring ecdysone is α-ecdysone or β-ecdysone.

17. (Reiterated) A method according to claim 15 wherein said ecdysone analog is ponasterone A, ponasterone B, ponasterone C, 26-iodoponasterone A, muristerone A, inokosterone or 26-mesylinokosterone.

18. (Reiterated) A method according to claim 15 wherein said ecdysone mimic is 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, a 1,2-diacyl hydrazine, an N'-substituted-N,N'-disubstituted hydrazine, a dibenzoylalkyl cyanohydrazine, an N-substituted-N-alkyl-N,N-diaroyl hydrazine, an N-substituted-N-acyl-N-alkyl, carbonyl hydrazine or an N-aroyl-N'-alkyl-N'-aroyl hydrazine.

19. (Reiterated) A method according to claim 1 wherein said exogenous gene is a wild type gene and/or therapeutic gene.

20. (Amended) A method according to claim 19 wherein said wild type gene is selected from genes which encode products:

the substantial absence of which leads to the occurrence of a non-normal state in said cell; or

a substantial excess of which leads to the occurrence of a non-normal state in said cell.

21. (Amended) A method according to claim 19 wherein said therapeutic gene is selected from those which encode products:

which are toxic to the cells in which they are expressed; or

which impart a beneficial property to said cells.

22. (Twice Amended) A method of inducing the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising an exogenous gene under the control of an ecdysone response element,

(iii) DNA encoding a modified ecdysone receptor under the control of an inducible promoter; wherein said modified ecdysone receptor, in the presence of a ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element, and

(iii) a ligand for said modified ecdysone receptor;

said method comprising subjecting said cell to conditions suitable to induce expression of said modified ecdysone receptor.

23. (Twice Amended) A method of inducing expression of an exogenous gene in an isolated cell containing a DNA construct containing said exogenous gene under the control of an ecdysone response element, said method comprising introducing into said cell:

a modified ecdysone receptor; and

a ligand for said modified ecdysone receptor,

wherein said receptor, in combination with a ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element, activating transcription therefrom.

24. (Twice Amended) A method for the expression of a recombinant product detrimental to isolated host cells, said method comprising:

transforming suitable isolated host cells with:

(i) a DNA construct encoding said recombinant product under the control of an ecdysone response element, and

(iii) DNA encoding a modified ecdysone receptor;

growing said host cells in suitable media; and

inducing expression of said recombinant product by introducing into said host cells ligand(s) for said modified ecdysone receptor, and optionally a receptor capable of acting as a silent partner for said modified ecdysone receptor.

35. (Reiterated) A method according to claim 4, wherein said member of the steroid/thyroid hormone superfamily of receptors is selected from: EcR, vitamin D_3 receptor, RARα, RARβ, RARγ, RXRα, RXRβ, RXRγ, TRα, TRβ, or ER.

36. (Reiterated) A method according to claim 35, wherein the DNA-binding domain of the modified ecdysone receptor is characterized as having a P-box amino acid sequence that differs from the P-box amino acid sequence of the naturally occurring DNA-binding domain.

37. (Reiterated) A method according to claim 36, wherein said modified P-box amino acid sequence preferentially binds to a different hormone response element half-site than said naturally occurring P-box amino acid sequence.

38. (Reiterated) A method according to claim 37, wherein the DNA-binding domain of said modified ecdysone receptor is derived from EcR and the P-box amino acid sequence is GSCKV (SEQ ID NO:3).

39. (Reiterated) A method according to claim 13, wherein said first half-site is obtained from an ecdysone response element and said second half-site is obtained from a hormone response element selected from a glucocorticoid response element, a mineralocorticoid response element, a progesterone response element or an androgen response element.

40. (Reiterated) A method according to claim 39, wherein said first half-site is obtained from an ecdysone response element and said second half-site is obtained from a glucocorticoid response element.

41. (Reiterated) A method according to claim 40, wherein said first half-site is AGTGCA and said second half-site is TGTTCT.

42. (Reiterated) A method according to claim 13, wherein said ecdysone response element has the sequence AGTGCA-N-TGTTCT.

47. (Reiterated) A method according to claim 1, wherein said receptor capable of acting as a silent partner is present.

48. (Reiterated) A method according to claim 47 wherein said receptor capable of acting as a silent partner is ultraspiracle.

49. (Reiterated) A method according to claim 1 wherein said modified ecdysone receptor has substantially no binding affinity for endogenous response elements.

50. (Amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element;

said method comprising providing to said cell an effective amount of a ligand for said modified ecdysone receptor; wherein said ligand is not normally present in said cell; and wherein said ligand is not toxic to said cell.

51. (Reiterated) A method according to claim 50, wherein said receptor capable of acting as a silent partner is RXR.

52. (Reiterated) A method according to claim 50, wherein said receptor capable of acting as a silent partner is ultraspiracle.

53. (Amended) A method for modulating the expression of an exogenous gene in an isolated mammalian cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element;

said method comprising providing to said mammalian cell an effective amount of a ligand for said modified ecdysone receptor; wherein said ligand is not normally present in said mammalian cell; and wherein said ligand is not toxic to said mammalian cell.

54. (Reiterated) A method according to claim 1, wherein said receptor capable of acting as a silent partner is present.

55. (Reiterated) A method according to claim 54, wherein said receptor capable of acting as a silent partner is RXR.

56. (Reiterated) A method according to claim 54, wherein said receptor capable of acting as a silent partner is ultraspiracle.